



Desalting and Buffer Exchange by Dialysis, Gel Filtration, or Diafiltration*

By Larry Schwartz, Senior Technical Manager, Pall Life Sciences

*Rewritten from the article *Buffer Exchange By Gel Filtration or Diafiltration* by John Rozembersky and Ron Spears.

Dialysis

Dialysis is an old established procedure for reducing the salt concentration in samples. It requires filling a dialysis bag (membrane casing of defined porosity), tying the bag off, and placing the bag in a bath of water or buffer. Through diffusion, the concentration of salt in the bag will equilibrate with that in the bath. Large molecules that can't diffuse through the bag remain in the bag. If the bath is water, the concentration of the small molecules in the bag will decrease slowly until the concentration inside and outside is the same. The greater the volume of the bath relative to the sample volume in the bags, the lower the equilibration concentration that can be reached. Usually several replacements of the bath water are required to completely remove all of the salt. The final volume in the bag is similar to the starting volume though it may have changed a little due to osmotic effects. Once equilibration is complete, the bag is ruptured and the solution poured off into a collection vessel.

Dialysis is quite slow, taking as much as several days for almost complete salt removal. It also requires manual manipulation of the dialysis bags for filling and tying off. Many samples have ended up on the floor as a result of bags breaking or slipping out of hand while tying. While dialysis can be used for volumes up to a few liters, it is not practical for larger sample volumes.

Gel Filtration

Gel filtration is a non-adsorptive chromatography technique that separates molecules on the basis of molecular size. Desalting and buffer exchange are two special examples of gel filtration that are widely used in many downstream bioprocesses. Desalting is used to completely remove or lower the concentration of salt or other low molecular weight components in the sample while buffer exchange replaces the sample buffer with a new buffer.

Gel filtration is one of the easiest chromatography methods to perform because samples are processed using an isocratic elution. In its analytical form, gel filtration (also known as size exclusion chromatography) can distinguish between molecules (e.g. proteins) with a molecular weight difference of less than a factor of 2 times. In this application, the porosity of the gel filtration media to be used is selected to provide high resolution in the molecular weight range of interest.

The more common applications for gel filtration are desalting and buffer exchange. In these applications, the size difference between the substances being separated is very large (i.e. proteins vs. salts). A gel filtration media is chosen that completely excludes the larger molecules while allowing the smaller molecules to freely diffuse into all of the pore spaces. The column is equilibrated with a buffer, which may be the same or different from that of the sample. Following application of the sample to the column, more of the column buffer (eluting buffer) is added to carry the sample molecules down the column. The larger molecules, which can't enter the pores of the media, elute first from the column, followed by the smaller molecules that diffuse into the pores, slowing them down relative to the larger molecules. If the eluting buffer is different from the sample that was applied, the larger molecules will be displaced from the original salts and elute in this new buffer, completely separated from the original sample buffer.

One important aspect of gel filtration is that the applied sample will be diluted as it elutes. The dilution factor depends on the volume of sample applied relative to the size of the column. The smaller the sample volume applied to the column, the greater the dilution factor. At best, the smallest dilution is approximately 1.5 times, if a sample load equal to 1/3 of the column volume is applied. This is the maximum volume of sample that can be applied. If a greater volume is applied, there will be overlap between the large and small molecules and complete separation will not be achieved. Large sample volumes require very large gel filtration columns. It is also possible to use a smaller column and apply an aliquot of the sample. In this case, many cycles may have to be run to process the entire sample volume. The individual large molecule fractions from each run can then be pooled.

The final process volume will always be greater than the starting volume. Large columns are expensive and require a significantly large footprint. For this reason, gel filtration for buffer exchange is often used in the later steps of downstream processing where sample volume has been reduced.

Gel filtration is simple to run, but requires operator manipulation (or automation). Material requirements include a reliable buffer delivery system, and tanks to hold the buffer needed for the procedure. An ultraviolet wavelength detector is usually employed to detect when the sample elutes from the column. A conductivity detector is used to detect the salt peak and measure the effectiveness of the separation.

Since the media used is generally based on hydrophilic porous beads made from dextran or agarose polymers, good product recoveries (> 95%) can usually be expected for hydrophilic biomolecules. In addition, well-designed columns offer little resistance to fluid flow keeping product shear down to a minimum. Depending on the media chosen and column dimensions, desalting is relatively fast compared to using gel filtration for fractionating molecules that are relatively close in molecular size. Separation times under 15 minutes are common.

In summary, gel filtration offers good product recovery and simple process operation. It is widely used for desalting and buffer exchange throughout the biotechnology industry and has proven to be predictable and reliable. However, this method does have some significant limitations.

Diafiltration

An alternative technique for desalting or buffer exchange is diafiltration. Diafiltration is an adaptation of a filtration technique more commonly known as ultrafiltration.

Ultrafiltration is an established separation process that has been an effective industrial operation for over 25 years. The process selectively utilizes permeable membrane filters to separate the components of solutions and suspensions based on their molecular size. Ultrafiltration has been widely used for the concentration, diafiltration, or fractionation of mixtures of biomolecules.

In ultrafiltration, the solution to be processed is brought in contact with a membrane. The membrane chamber is pressurized either by gas or by use of a positive displacement pump. The liquid and solutes, whose size is smaller than the membrane pores, pass through the membrane. This solution is known as the filtrate or permeate. Molecules in solution that are larger than the pores in the membrane are excluded from the pores and are retained by the membrane. This solution is known as the concentrate or retentate.

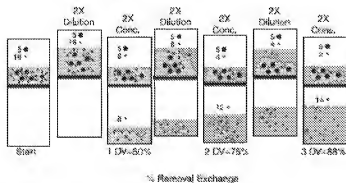
If the goal is to remove all the low molecular weight materials from the sample (e.g. desalting), and perhaps to also exchange the starting sample buffer salt with a different buffer, then simple concentration is insufficient. Assuming that the buffer salt is 100% permeable and buffer salts and liquid are removed as the product concentration increases, the concentration of salt in both the filtrate and retentate remains the same. To reduce the concentration of salts, solvents, or other low molecular weight species, diafiltration must be performed.

The technique of continuous diafiltration involves washing out the original buffer salts (or other low molecular weight species) in the sample by adding water or a new buffer to the sample at the same rate as filtrate is being generated. If water is used for diafiltration, the salts will be washed out and the conductivity lowered. If a buffer is used for diafiltration, the concentration of the new buffer salt in the sample will increase at a rate inversely proportional to that of the species being removed.

Using continuous diafiltration, greater than 99.5% of a 100% permeable solute can be removed by washing through six equivalent sample volumes with the buffer of choice.

Diafiltration can also be performed in a discontinuous mode by first diluting the sample with an equal volume of water or the new buffer, and then concentrating back to the original volume. An example is shown in Figure 1.

Figure 1
Discontinuous (Sequential Dilution) Diafiltration



Each additional diafiltration volume further reduces the concentration of remaining salt.

The following table gives the percent of salts in the permeate after the indicated number of diafiltration volumes have been washed through the membrane.

Table 1
Salt Removal by Diafiltration

Volumes	Continuous	Discontinuous
1	63.2	50.0
2	86.5	75.0
3	95.0	87.5
4	98.2	93.8
5	99.3	96.9
6	99.8	98.4
7	99.9	99.2

A diafiltration volume is the volume of product (concentrate) at the start of diafiltration.

Note that it takes fewer diafiltration volumes using continuous diafiltration compared to discontinuous diafiltration to remove the same amount of salt.

It is possible to optimize the process relative to time and process volume because both concentration and diafiltration are performed on the same membrane and equipment. By concentrating a sample before diafiltration, a much smaller volume is required for diafiltration. For example, starting with 1 liter of product, it takes 1 liter (1 DV) of diafiltration buffer to remove 50% of the salt by discontinuous diafiltration. If the product were first concentrated 10x to 100 mL, then it would only require 100 mL of diafiltration buffer to remove 50% of the salt. Note that in both cases the final salt concentration in the sample (concentrate) is the same. However, the concentrated solution will be more viscous. Actual viscosity is dependent on the characteristics of the specific molecules that make up the sample. This viscosity effect becomes very significant as the product concentration increases above a few percent. With increased viscosity, the filtrate flux rate will be lower. Although the process volume is reduced 10 fold, the time will not be reduced proportionately.

Simple protocols are available to find optimum conditions to maximize productivity.

In diafiltration, the filtrate flux rate remains relatively constant during the process except if the sample viscosity changes due to concentration effects or if changes in the ionic environment change the conformation of a retained molecule and its permeability.

Dialysis, Gel Filtration, or Diafiltration?

With readily available gel filtration columns or membrane devices, there is little reason to use dialysis. Diafiltration can easily be substituted for gel filtration applications such as desalting, buffer exchange, or even removal of dilute solvents. The question remains as to whether or not it is desirable to consider such a substitution. With proper process design (selection of membrane by pore size, use of proper pump and membrane holder, etc.), diafiltration can be a rapid and efficient method of desalting. Furthermore, scaling up the process can often be as simple as adding more membrane to the system. Membranes can be easily cleaned and reused for long periods and multiple process cycles. Chromatography columns can be cleaned and reused but often become fouled and require repacking. Membrane systems can be easily scaled to process large volumes of sample as one batch by varying the membrane area or process time. Chromatography columns are limited by the amount of sample load and cost. Big columns can be expensive and difficult to work with. For large sample volumes, gel filtration columns are chosen so multiple cycles using smaller volumes of sample must be run to process the entire batch. The resulting product from each cycle is then pooled back into one batch.

Continuous diafiltration has a distinct advantage, unlike gel filtration, because the volume of the sample remains constant throughout the process. Furthermore, after the diafiltration is complete, the product can be further concentrated in the same system. This is an effective combination of two process steps. If desalting by gel filtration is performed, the product is diluted, typically by 40% or greater, and often must be further concentrated by ultrafiltration. Thus, using ultrafiltration for both

desalting and concentrating completely eliminates the need for the gel filtration column.

Summary

Dialysis is an old technique that can be replaced by gel filtration or diafiltration. Diafiltration can be used in place of gel filtration for desalting, buffer exchange, or solvent removal. It is economical and easy to perform. The ability to concentrate the product following diafiltration further increases the utility of the ultrafiltration system and increases the value of such a system to a production facility that uses it.